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(54) CULTIVATION OF FILAMENTOUS FUNGI

(71) We, THE UNIVERSITY of STRATHCLYDE, incorporated by Royal Charter, of Royal College, 204 George Street, Glasgow, G1 1XW, Scotland, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a method of cultivating filamentous fungi under aerobic conditions.

When fungi are cultivated under aerobic conditions the necessary oxygen may be supplied in various ways. In the known process for "deep-culture" method oxygen gas or air is forced in fine bubbles through a liquid nutrient containing the fungi. In the known "koji" method the nutrient and fungi are simply spread on a tray where they lie in contact with ambient air.

The effect of changes in growth conditions upon the production of metabolites or products by fungi is in general not readily predictable and novel conditions often produce results which cannot be anticipated.

The present invention is concerned with the supply of oxygen to growing fungi in a liquid nutrient medium. One of the disadvantages of the deep-culture method is that filamentous fungi tend to adhere to the wall surfaces of the container used and excessive agitation is necessary to keep them in suspension. Apart from the excessive energy demands of the agitation the mechanical agitation disintegrates the filamentous growth.

The present invention utilises this adhesive property of filamentous fungi, hitherto considered to be disadvantageous, to provide a new method for the aerobic cultivation thereof.

According to the present invention there is provided a method of cultivating filamentous fungi comprising partially filling a rotating disc cultivator with a liquid nutrient medium for the fungi, introducing sterile air into the cultivator in the space above the liquid nutrient medium, inoculating spores of the fungi into the nutrient and permitting said spores to adhere to the discs of the cultivator, rotating the discs whereby the spores adhering thereto are cyclically subjected to the nutrient and the sterile air and grow to form a layer of fungi adhering to the discs, wherein one or more disc is segmented and the segments are manually removable from the cultivator under sterile conditions to permit monitoring of the growth of the fungi.

Preferably the nutrient medium is a flowing stream of liquid.

It is clearly advantageous to select the material of the plate to obtain optimum adhesion of the culture thereto, for example, fungal strains such as those of the species of the *Mucor, Rhizopus, Lasiodiplodia* and *Aspergillus genera,* adhere poorly to glass but adhere well to substances such as compressed asbestos or synthetic plastics material.

It is also preferred that the vessel be made of materials that will withstand heat sterilisation.

An embodiment of the invention will now be described, by way of example, with reference to the accompanying drawing.

Apparatus for cultivating filamentous fungi in accordance with the method of the present invention has a horizontally disposed cylindrical vessel 10 constructed of a glass tube having a pair of end-plates 11, 12 of synthetic plastics material. The end-plates are secured by means of bolts 13 extending, externally of the glass tube, between the end-plates. A rotatable shaft 14 extends axially through the vessel, an aperture being provided in the end-plate 12 for the shaft and a recess being provided in the end plate 11 for receiving and bearing the shaft end. A seal and bearing 15 is provided for the shaft 14 where it passes through the aperture in the end-plate 12 into the vessel.

A number of circular discs 18 are mounted centrally on the shaft, each disc acts as a carrier plate for the fungi and is separated from an adjacent disc by means of spacers 18 in the form of sleeves on the shaft.

A low-level inlet 20 and a corresponding outlet 21 is provided in the end-plates to permit throughflow of liquid and a high-level inlet

22 and corresponding outlet 23 permits through-flow of sterile oxygen-containing gas in the opposing direction to nutrient flow.

An electric variable-speed drive motor 30 is provided for rotating the shaft.

In performing the method of the invention the vessel is first loaded with clean carrier plates, a liquid nutrient medium is passed into the vessel until it flows from the liquid outlet 21, a circulating flow of nutrient may then be established if desired, but a body 32 of the nutrient medium is established in the vessel 10.

A flow of sterile air is passed through the vessel 10 via the inlet 22 and outlet 23.

The body 32 of nutrient medium is then inoculated with spores of the fungi to be cultivated. Initially the fungi may be allowed to grow without rotation of the discs 18 until the nutrient supply of the body 32 is depleted. The discs 18, on rotation, lift the adhering fungi out of the nutrient body 32 and into contact with the flow of air. As the discs thus rotate the culture is effectively oxygenated.

To facilitate monitoring of the growth, the apparatus may include a sampling device in the form of a radially segmented plate releasably attached to the shaft 14 by spring clips. The apparatus may have an air-lock system fitted as a side arm to the vessel 10 and through which individual segments of the segmented plate may be withdrawn, from time to time, by hand. There may be several such segmented plates, the side arm is sealed to one end of a flexible tube which can be sealed between its ends. Thus samples removed from the interior of the vessel 10 can be moved to the free end of the flexible tube which can then be sealed to permit removal of the sample therein from the other end.

The extent of the growth on the discs 18 can be determined by visual inspection through the vessel 10 and the constitution of the nutrient body 32 may be determined analytically by withdrawal of a sample therefrom.

The method of the present invention has produced unexpected results, for example the fungal organism *Lasiodiplodia theobromae* grown by conventional surface (koji) culture is known to produce small quantities of gum. With an identical nutrient medium it was found that substantially larger quantities of gum were produced by the method of the present invention. In one such experiment surface (koji) culture yielded 0.002 gm gum per gm dried weight of fungal mycelium, the corresponding figure for the present method being 0.03 gm gum per gram dried weight of fungal mycelium.

The apparatus may thus be used for continuous fermentation, products of interest being recovered from the nutrient body 32 and appropriate amounts of fresh nutrient added from time to time.

If optimal growth is not required the method may be used for batch culture instead of continuous culture.

Also, the mycelium may be recovered by removal from the plates mechanically.

After completion of a run, the apparatus may be sterilised either by dismantling and autoclaving the parts or by passing steam through the vessel 10 without dismantling.

The discs 18 used in the apparatus may be interchangeable so that the same apparatus with discs of different materials may be used for fungi having differing adherent characteristics. The speed of revolution of the discs 18 is preferably controllable so that maximal aeration with minimal mechanical stress may be applied according to the growth characteristics of the particular fungus.

The method according to the invention thus utilises the tendency of filamentous fungi to attach to surfaces. The system of continuous nutrient flow and removal provides a convenient method of cultivation on both laboratory and commercial scale.

The apparatus permits the growth in pure culture of filamentous fungi which have surface-adherent characteristics and may be used for the study of such fungi or for the production from them of desired products, either intracellular or secreted into the medium. Such products may be small molecules such as antibiotics or citric acid or large molecules such as enzymes.

By way of example, filamentous fungi may first be cultivated on the rotating discs in the presence of a liquid nutrient which is selected for optimal growth of the particular fungi ("a nutrient-rich medium"). Then when adequate growth has been achieved the nutrient is replaced by a medium which lacks growth-promoting characteristics appropriate to the fungi ("a nutrient-deficient medium") but which contains a solute convertible by the fungi. The fungi, then in a state of suspended growth carries out a chemical conversion of solute in the medium which may be removed in batches or semi-continuously. Citric acid may be produced from a sucrose solution utilising this process. In one production process for citric acid a strain of the filamentous fungi *Aspergillus niger* was grown for three days using a simple fermentation medium held at 28 °C. The nutrient-rich medium was then replaced by a nutrient-deficient medium lacking in nitrogen and phosphate sources. Over a period of five days the weight of fungus remained constant but continued to convert sucrose to citric acid, 70% conversion being finally obtained. By way of comparison the same conversion carried out by the deep culture method did not exceed 10%.

For aerobic cultivation of filamentous fungi the present method has advantages over the deep culture method in that adherence of the fungi to surfaces (which makes some fungi difficult or impossible to work with in deep culture vessels) is exploited as a useful feature. A continuous flow, batch-organism system, unlike deep culture, does not require continuous

removal of the fungi. Such removal of fungi may be unavoidable in deep-culture fermentations because of the necessity to remove liquid medium either for elimination of accumulated toxic substances or for collection of soluble products. The use of the method described here for production of fermentation products has a major advantage in that dissolved products do not have to be separated from the suspended fungi.

Again, in conventional continuous culture it is necessary to supply media both for conversion to product and for synthesis of fresh cells to replace those inevitably washed out. In the present method described here, since all biomass is retained, continuous synthesis of the fungi during product conversion is not necessary.

It is also the case that in conventional deep-culture continuous fermentation with filamentous fungi, a major problem is bacterial contamination because the bacterial growth rate exceeds that of fungi. In the rotating disc cultivator described above, washout of the nutrient body operates selectively against contaminants.

Similarly, in the continuous deep culture of filamentous fungi there is a selection of faster growing strains often to the detriment of the desired properties possessed by the original strain.

The method described herein does not depend on sustained reproduction to balance loss of fungi in washout but makes it possible to retain the biomass of the original strain since by adjustment of the nutrient medium constituents it is possible to maintain viability for product formation without promoting fungal growth.

The method has advantage over the tray (koji) culture method in ready control of parameters, such as pH.

The continuous nature of the process facilitates collection of metabolites and potentially useful products from the fungi.

The method provides for studying surface growth of fungi in pure culture under conditions which differ from those of existing methods. Large models for industrial purposes have the advantage over deep culture methods of using low power input.

This method of the present invention in general offers a ratio of surface growth to nutrient medium volume which is very high but can readily be varied by selection of the disc spacers 19. Since the fungi in pure culture are attached, a change in the carbon/nitrogen ratios in the nutrient medium can be rapidly achieved if such change should be desirable for optimal production of enzymes or other metabolites.

The method offers environmental conditions close to natural for the growth of surface-residing fungi. Compared to deep culture the mycelia are free from the effect of shearing forces which are often undesirable.

WHAT WE CLAIM IS:–

1. A method of cultivating filamentous fungi comprising partially filling a rotating disc cultivator with a liquid nutrient medium for the fungi, introducing sterile air into the cultivator in the space above the liquid nutrient medium, inoculating spores of the fungi into the nutrient and permitting said spores to adhere to the discs of the cultivator, rotating the discs whereby the spores adhering thereto are cyclically subjected to the nutrient and the sterile air and grow to form a layer of fungi adhering to the discs, wherein one or more disc is segmented and the segments are manually removable from the cultivator under sterile conditions to permit monitoring of the growth of the fungi.

2. A method as claimed in claim 1, where the discs are mounted coaxially on the rotatable shaft of the cultivator, extend vertically upwards from the liquid nutrient medium and the shaft is mounted horizontally.

3. A method according to any preceding claim in which the fungi are of the *Mucor, Lasiodiplodia, Rhizopus or Aspergillus* genera.

4. A method of cultivating micro-organisms, according to any one of the preceding claims, substantially as hereinbefore described.

5. A method of producing microbiological products by conversion of organic matter comprising applying a culture of filamentous fungi to the discs of a rotating disc cultivator and cyclically subjecting said culture to sterile air and to a nutrient medium containing growth-promoting constituents thereby to produce an adherent layer of the fungi on the discs, replacing the nutrient with a substrate deficient in growth-promoting constituents and containing material convertible by the fungi, cyclically subjecting the disc-adherent culture to sterile air and the said substrate whereby the convertible material is converted by the fungi to a product and recovering said product from the substrate.

6. A method of producing citric acid, comprising applying a culture of the fungus *Aspergillus niger* to the discs of a rotating disc cultivator and cyclically subjecting the culture to liquid nutrient medium containing growth-promoting constituents for the fungus and to sterile air thereby to produce on the discs an adherent culture of the fungus, replacing the nutrient medium with a substrate deficient in growth-promoting constituents and containing sucrose, cyclically subjecting the disc-adherent culture to sterile air and to the substrate thereby to convert the sucrose to citric acid, and recovering the citric acid, from the substrate.

7. A method of producing microbiological products, according to claim 5 or 6 substantially as hereinbefore described.

8. Apparatus for carrying out the method claimed in claim 1, comprising a rotating disc cultivator having a generally cylindrical vessel disposed on a horizontal axis, a rotatable shaft extending coaxially through the vessel, a

plurality of circular discs mounted coaxially on the shaft, means for introducing liquid into and maintaining a body of liquid in the vessel, means for supplying sterile air to a space above the liquid and means for rotating the shaft and discs, and including at least one segmented disc releasably mounted on the shaft and means for manually removing segments of the said disc from the vessel under sterile conditions.

9. Apparatus according to claim 8 in which means are provided for circulating the liquid through the vessel.

10. Apparatus according to claim 8 or 9, in which the vessel has a side arm having therein an air-lock assembly through which the disc segments may be withdrawn.

11. Apparatus as claimed in claim 8, substantially as hereinbefore described with reference to and as illustrated by the accompanying drawing.

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1581832 COMPLETE SPECIFICATION

1 SHEET *This drawing is a reproduction of the Original on a reduced scale*